

# **The Effects of Dental Pulp Derived Stem Cells on the Interleukin Response in Equine Osteoarthritis**

Honors Research Thesis

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Distinction

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## **Abstract**

Intra-articular use of dental pulp stem cells may have the potential to decrease lameness and joint inflammation in naturally occurring osteoarthritis in horses. The goal of this project was to investigate the effects of dental pulp derived stem cell therapy on the immune and inflammatory response, specifically cytokine profiles, when administered intra-articular to horses with osteoarthritis. Twenty horses were randomly assigned to receive 1ml of control solution (n=10) or 10 million cells of dental pulp (n=10), exercised on a treadmill, and synovial fluid evaluated before and at day 14 after injection for cytology and IL-1 $\beta$ , IL-1 receptor antagonist, IL-6, and IL-10 [Genorise ELISA kit]. Data was analyzed by repeated measure ANOVA for time and treatment and Wilcoxon rank post-test. Statistical significance was set at  $P < 0.05$ . Synovial fluid WBC count, protein and cell differentials at Day 0 or 14 did not differ between treated and control and synovial fluid was noted as within acceptable limits with counts  $> 1000$  cells/ul were only seen in samples with blood contamination. The IL-10 percent change from baseline differed and increased in the control joints and decreased in the treated joints. ( $P < 0.03$ ) The control synovial fluid increased in IL-6 ( $p < 0.03$ ) and IL-6 percent change from baseline increased in the control synovial fluid and decreased in the treated synovial fluid. ( $P < 0.03$ ) No significant difference was noted between groups for IL-1 $\beta$  and IL-1 receptor antagonist and values were low. Adverse effects were not observed. Intra-articular injection of dental pulp therapy was not pro-inflammatory in the synovial fluid after 14 days and demonstrated persistent anti-inflammatory and immunologic effects as measured by cytokine analysis. Intra-articular administration of dental pulp derived stem cell therapy can be considered a safe treatment option for equine osteoarthritis, with the potential for disease modifying effects.

## **Introduction**

Osteoarthritis is a multifactorial degenerative disease with loss of articular cartilage components, due to an imbalance between extracellular matrix destruction and repair [Todhunter et al, 1996].

This disease can result in lameness, stiffness, joint swelling and decreased activity or performance. Autologous conditioned serum (ACS) is one treatment option for osteoarthritis with published support. Dental pulp has recently served as a source of pluripotent stem cells (DPC) with differentiation potential for bone, ligament, and, uniquely, neural tissue. [Waselau et al, 2008], [Bertone et al, 2014]. Intra-articular use of DPC may have the potential to decrease lameness and joint inflammation in naturally occurring osteoarthritis in horses. The objective of the study was to investigate the effects of dental pulp derived stem cell therapy (PulpCyte®, StemLutions LLC.) on the immune and inflammatory response, specifically cytokine profiles, when administered intra-articular to horses with osteoarthritis. Cytokine inhibitor directed therapy is believed to work by up-regulating the expression of beneficial cytokines such as IL-1Ra and limiting cytokines with negative affects seen in osteoarthritic diseases. Our hypothesis was that synovial fluid collected from horses treated with DPC would have lower levels of inflammatory cytokines when compared to the control group. If DPC treated horses had lower levels of inflammatory cytokines, this may indicate that DPC treatment impacts the progression of osteoarthritis.

## **Materials and Methods**

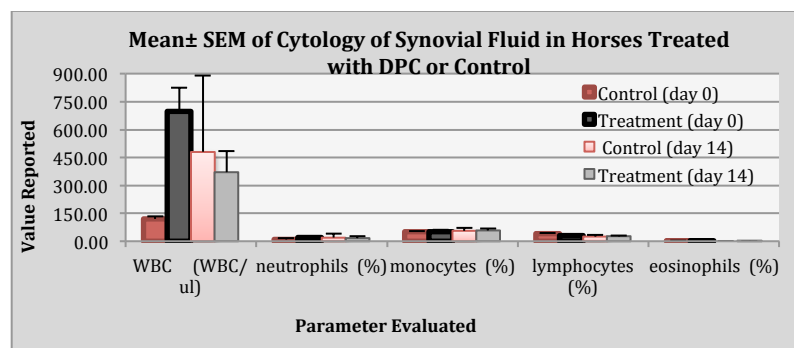
The study was designed as a randomized, double blind, placebo-controlled clinical trial. Twenty horses with confirmed osteoarthritis were randomly assigned to receive 1ml of control solution (n=10) or 10 million cells of dental pulp (n=10) and exercised on a treadmill once on day 0 of treatment and again on day 14 of treatment. Synovial fluid was collected from the joints at day 0 pre-injection and at day 14. The age of the horses in the study had a range of 2 years in the control group and 7 years in the treatment group. The average age was 16 years with a standard error of 2 years for the controls and 12 years with a standard error of 7 years for the treated. The weight of horses in the study ranged from 389 to 781 kg, with an average weight of 537 kg.

Eight breeds were represented in the study. The sample consisted of 14 mares, one stallion, and 25 geldings (castrated males). Collected synovial fluid was placed in a glass tube containing EDTA and in a plastic cryopreservation vial. Samples in EDTA were immediately evaluated for cytology and samples in the cryopreservation tubes were frozen at  $-80^{\circ}\text{C}$  until ELISA analysis. ELISA was performed in duplicate by use of commercially available kits for IL-6, IL-10, IL-1 $\beta$ , and IL-1 receptor antagonist [Genorise ELISA kit]. Data was analyzed by repeated measure ANOVA (Excel, Microsoft) for time and treatment and Wilcoxon rank post-test. Statistical significance was set at  $P < 0.05$ .

## Results

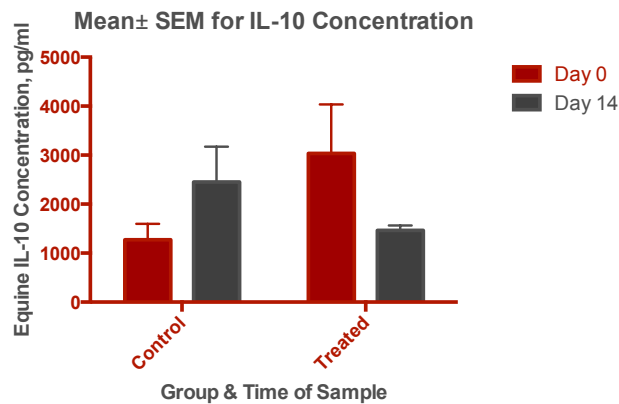
All synovial fluid samples were taken prior to injection on either day 0 or 14. In the synovial fluid from day 0, 50% of horses had sufficient sample across treatments. Sample was tested from 3 control and 7 treated horses. Two horses (1 control, 1 treated) presented neutrophilic inflammation. Also, 3 treated horses presented mild suppurative inflammation. In the synovial fluid samples from day 14, again 50% of horses had sufficient sample. Sample was tested from 3 control and 6 treated horses. Several samples had blood contamination. All synovial fluid WBC count, protein and cell differentials at day 0 or 14 [Figure 1] did not differ between treated and control. All synovial fluid was noted as within acceptable limits with counts  $> 1000$  cells/ul were only seen in samples with blood contamination.

Figure 1:



The IL-10 percent change from baseline [Figure 2] differed and increased in the control joints and decreased in the treated joints ( $P<0.03$ ).

Figure 2:



The control synovial fluid increased in IL-6 ( $p < 0.03$ ) and IL-6 percent change from baseline increased in the control [Figure 4] synovial fluid and decreased in the treated [Figure 3] synovial fluid ( $P<0.03$ ).

Figure 3:

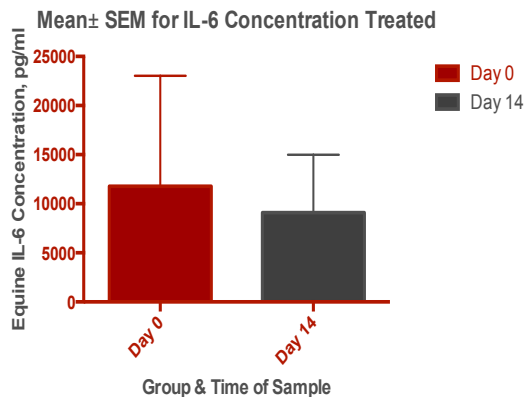
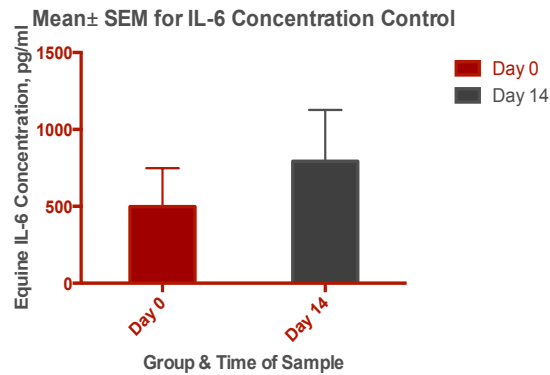


Figure 4:



No significant difference was noted between groups for IL-1 $\beta$  [Figure 5] and IL-1 receptor antagonist [Figure 6] and values were low. In all horses no adverse effects were observed.

Figure 5:

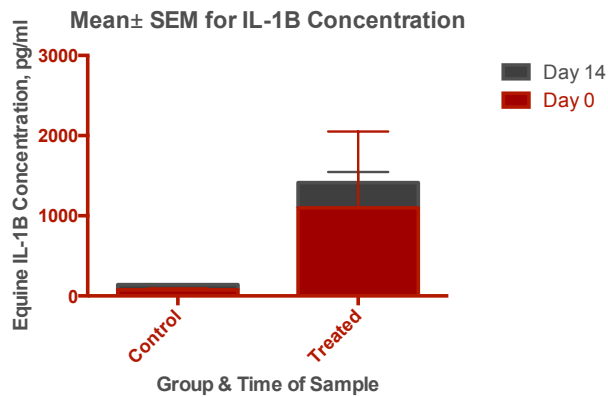
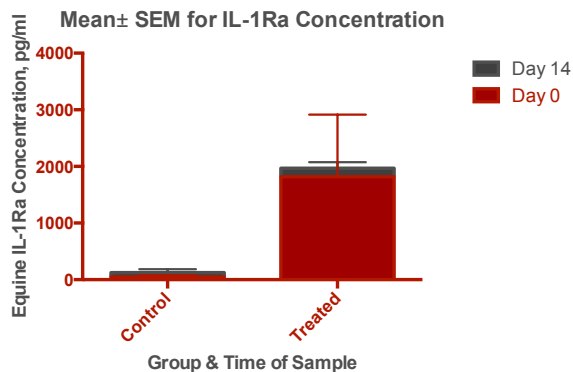


Figure 6:



## Discussion

Equine dental pulp stem cells (DPC) are a therapeutic product available for the treatment of degenerative conditions of the limbs in horses. Previous studies have shown that equine mesenchymal stem cells (MSC) delivered with platelet concentrates have the ability to assist with tissue repair due to their ability to differentiate into a variety of cell lines [Del Bue et al, 2008]. In regards to MSC and immunity, intra-articular injections of genetically modified xenogeneic bone marrow derived MSC have been shown to increase CD4+ lymphocytes and inflammatory cytokines in thoroughbred mares [Pigott et al, 2013]. Although the efficacy of stem cell injections for degenerative diseases has been evaluated in horses in previous studies, few studies have focused specifically on the equine immune response to this treatment. This study analyzed the immunomodulatory effects of DSCD PRP injections in horses presenting clinical lameness and how the immune response to injections factors into the efficacy of stem cell treatment. In this study, the problem of clinical lameness was defined as a spectrum of conditions within osteoarthritis, tendonitis, and desmitis. In the studied population, dental pulp stem cell therapy did not increase pro-inflammatory cytokines (IL-6, IL-1B) in the synovial fluid of osteoarthritic horses and it may have the ability to increase anti-inflammatory cytokines. Results were similar to a recent study in which intra-articular injection of autologous protein solution in horses with osteoarthritis did not cause a significant difference in IL-1 $\beta$  and IL-1 receptor antagonist levels between control and treatment groups [Bertone et al, 2014]. Intra-articular administration of dental pulp derived stem cell therapy can be considered a safe treatment option for equine osteoarthritis, with the potential for disease modifying effects such as decreasing IL-6, an inflammatory cytokine. Further research in dental pulp therapy with a larger population size could provide additional data regarding the equine immune response to this cell therapy. Further research could also attempt to analyze the effects of dental pulp therapy on other cytokine markers such as TNF- $\alpha$ .

## **Bibliography**

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